Preparation of Corn Products Endogenously Labeled with Zinc-65 for Use in Bioavailability Studies

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Four identifiable fractionated products were prepared from yellow dent corn intrinsically labeled with ^{65}Zn via nutrient solutions. Introduction of the radioactive zinc was delayed until just before the corn ear developed. Over 27% of the ^{65}Zn that was absorbed by the corn plants was translocated to the kernels. The excised full-fat germ fraction, representing 10.23% of the whole kernel weights, contained 56.38% of the ^{65}Zn activity. The endosperm–hull fraction contained the remainder of the ^{65}Zn present in the kernels. After simultaneous grinding and fat extraction, relative activities (μCi ^{65}Zn/g of tissue) varied considerably in the four fractions produced: defatted germ flour (219.35), defatted endosperm–hull (13.79), oil from germ (8.25), and oil from endosperm–hull (1.18). The relative abundance of zinc in these endogenously labeled products measured radiometrically was compared with nonradioactive zinc in commercial corn food products.

Zinc is an essential element required by both plants and animals. Biologically available zinc resources in humans are small and a dietary intake of 15 mg of zinc/day is now considered adequate for healthy adults. Additional daily quantities of 15 and 10 mg also are recommended during periods of pregnancy and lactation, respectively (National Research Council, 1974; Sandstead, 1973). Furthermore, the zinc exchange rate in humans is estimated at 6 mg of zinc/day (Richmond et al., 1962). The need is very evident for definitive studies to establish the availability of zinc originating from a multitude of food sources. Zinc in the diet may be unavailable, or other dietary constituents present in the gastrointestinal tract may reduce absorption. The nutritional and biochemical importance of zinc in animal and human diets has been reviewed in depth recently (Halstead et al., 1974), but much less is known about the bioavailability of zinc in food products (Oberleas et al., 1966).

The present study was conducted to prepare four identifiable fractions of whole corn intrinsically labeled with ^{65}Zn for subsequent use in animal feeding studies. The first phase dealt with the incorporation of ^{65}Zn into corn plants that were growing in nutrient solution. Nonradioactive corn plants that produced whole kernel corn for use as sample controls in the feeding studies were grown at the same time and under similar conditions.

The second phase dealt with the fractionation of the corn kernel. In addition to defatted corn germ flour and endosperm–hull fractions, the oils derived from these two fractions also could be important in feeding studies even though the quantities of zinc occurring in these oils would be minor.

These studies were conducted to provide fractionated products of corn kernels containing ^{65}Zn which had been acquired by the tissue via normal absorption and translocation processes. The bioavailability of the zinc in these intrinsically labeled samples will reflect accurately the bioavailability of the zinc in food products that incorporate one or more of the kernel fractions we prepared and will allow unambiguous comparison with the bioavailability obtained from studies with food products to which zinc has been added extrinsically.

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Table I. Mineral Composition of Full-Strength Nutrient Solution[a] Used for Both Nonradioactive and Radioactive Corn

Element	Concentration, mM	Element	Concentration, μM
Ca	2.54	P	64.57
K	1.74	Fe	35.72
N (as NH_4)	0.93	Mn	6.61
N (as NO_3)	7.71	B	18.39
Cl	0.42	Zn	1.71
S	0.50	Mo	0.70
Mg	0.62	Cu	0.45

[a] Formulated according to R. B. Clark, private communication. For radioactive corn, the addition of 0.5 mCi ^{65}Zn (sp act. 8.2 Ci/mg) per liter per week increased the total Zn concentration by less than 1 nM.

MATERIALS AND METHODS

Apparatus. Translocation of the radioactive zinc from the nutrient solution to the specific corn plant tissues was measured from counts obtained from the ^{65}Zn 1.11 MeV photopeak. This was accomplished in a custom-built total body counter at the Human Nutrition Laboratory, Grand Forks, N. Dak., and also with a Nuclear-Chicago Model 1085 γ-ray spectrometer apparatus with a 5-cm NaI(Tl) crystal detector at North Dakota State University, Fargo, N. Dak. Ratemeter (Victoreen 440) readings were used for quantitation of the radiolabel in the corn fractions derived from the corn kernels. A standard containing 5.66 μCi ^{65}Zn was used for calibration of the counters.

The apparatus used for grinding–defatting operations consisted of a closed stainless-steel chamber with impeller and rotary cutting blades located at the base. The Waring explosion-proof power unit, Model 8830, was placed in a hood and controlled by a variable transformer and a timer located at a distance of 1 m outside the hood. Cooling water was circulated during the grind operation through an internal cavity at the base of the chamber.

Plant Culture, Treatments, and Harvest. Kernels of corn (*Zea mays* L., Oh43 × W64A) were germinated in paper rolls in the dark at 25 °C for 6 days (Blankendaal et al., 1972). Seedlings were then selected for uniformity and two were transplanted to each of a dozen 12-L containers (Osmond and Clark, 1970), each containing 10 L of nutrient solution (Table I), which was aerated continuously during the entire growth period. The plants were grown to maturity in the greenhouse under the natural photoperiod (13 to 15 h) with day and night temperatures averaging 33 and 23 °C, respectively. The nutrient solution

used initially was half-strength, and this solution was later replaced with a 70% strength solution 9 days after transplanting. This solution in turn was totally replaced with sufficient new full-strength nutrient solution at weekly intervals thereafter. Solution volume lost as a result of transpiration and aeration was maintained by adding deionized water as needed.

When tassels began to emerge (45 days after transplanting), two corn plants growing in one container and designated plants 1 and 2 were selected for the incorporation of the radioactive zinc. The experiment was designed so that approximately 5 mCi ^{65}Zn (carrier-free as the chloride salt) was added each week for 5 weeks with full-strength nutrient solutions. A cumulative total of 24.97 mCi ^{65}Zn as of July 1, 1975, t_0, was supplied. Each week, vials containing 5 mCi carrier-free ^{65}Zn (8.2 Ci/mg) in 2 mL of 1 N HCl were submerged and rinsed in the required nutrient solution. This solution was stirred thoroughly before corn plants 1 and 2 were transferred into it. Solution pH remained below 6 after ^{65}Zn was added. The used nutrient solutions were pooled weekly into a single container and assayed for remaining ^{65}Zn content.

The plants matured, the lower leaves became desiccated 100 days after transplanting, and the nutrient solution was removed from 11 nonradioactive containers at this time to allow the plants to air-dry. Ears from the nonradioactive corn plants were separated, and the other vegetative tissues were discarded 117 days after transplanting. However, all tissues from radioactive corn plants 1 and 2 were retained, separated, and weighed before subsequent ^{65}Zn quantitation measurements were made. Both radioactive and nonradioactive whole kernel corn was shelled from the cobs 5 days after harvest. Radioactive plants 1 and 2 yielded 93.5 g of air-dried kernels; the other 22 nonradioactive plants yielded over 450 g of kernels. The reduced yield per nonradioactive plant was accounted for by incomplete ear filling that resulted from reduced pollination of plants at the edge of the plant array.

Quantitation of ^{65}Zn Activity in Corn Tissues. Except for the roots, ^{65}Zn activity in the vegetative tissues for plants 1 and 2 was established by counting the subdivided tissues in the large whole body counter with the counting geometry adjusted to accommodate all samples. The activity of the roots was determined under specific geometric conditions, by intercomparing ratemeter readings with other vegetative tissues whose activity had previously been determined.

The radioactive whole-kernel corn sample represented 418 kernels. Of this sample, 22 individual kernels having a mean weight of 0.2309 ± 0.0362 g each were counted in the Nuclear-Chicago counter to establish their activities. Six of these same kernels were also counted in the whole body counter and the activities correlated (R = 0.9969). It was established that ^{65}Zn activity was 4982 μCi for the total whole-kernel sample, with an associated activity of 53.287 μCi ^{65}Zn/g of tissue as of t_0.

Composition of Nonradioactive Corn. The composition of the nonradioactive corn sample was determined by grinding 50 g of the kernels to pass through 40 mesh for the following analyses: protein, fat, ash, and fiber content as determined by standard methods (AACC, 1962). A value of 6.25 was used to convert nitrogen content to protein content. Starch was measured polarimetrically with 90% dimethyl sulfoxide as the solvent. A Bendix electronic polarimeter equipped with a 0.5-dm cell was used for optical rotation measurements at 546 nm (mercury green) (Garcia and Wolf, 1972). Pentosans were determined by a volumetric bromine method (AACC, 1962),

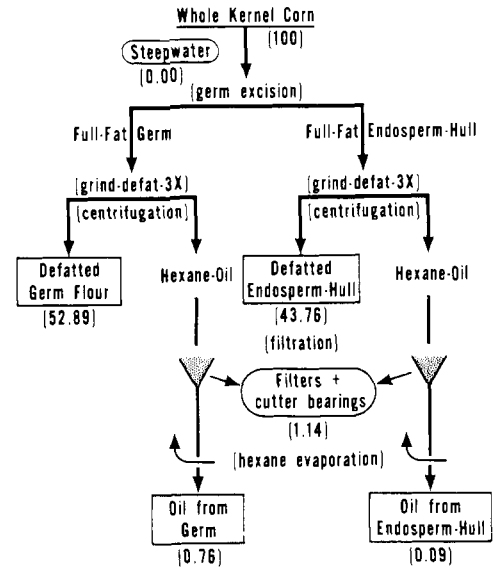

Figure 1. Flow diagram for processing radioactive and nonradioactive whole-kernel corn into four corn fractions. Encircled products were removed for radioactive disposal, where applicable. Values in parentheses represent the percent distribution of the ^{65}Zn in the fractions. An additional 1.36% of the activity was accounted for in the NaEDTA–Na_2CO_3 solution used to decontaminate the apparatus.

where furfural from the sample was distilled from 12% HCl. Zinc content was determined by flame atomic absorption after the sample had been wet-ashed with nitric acid.

Corn Fractionation. The radioactive whole-kernel corn sample, equally divided into four separate plastic bags, was stored in a 5-cm lead shield with an internal cavity of 20.4 cm^3. The radioactive corn was stored in this condition until after the necessary operations required for the excision of the germ and the subsequent grind and defat procedures had been optimized with the nonradioactive corn sample.

Dissection. The fractionation techniques that we used for both nonradioactive and radioactive whole-kernel corn samples are shown in the flow diagram (Figure 1). Groups of 15 to 20 kernels were transferred to tared screw-cap glass vials and weighed. Distilled water was added to these subdivided samples in a series of vials, and after at least a 4-h steeping period, the water was drained and cumulatively collected in a polyethylene bottle. The vials containing the radioactive kernels were stored in a lead shield. Individual kernels were removed with tongs for excision of the germ. With stainless-steel surgical tools, the germ with adhering pericarp was excised and transferred to a second preweighed glass vial in a second shield. The remaining portion of the kernel representing the endosperm–hull fraction was transferred to a third vial stored in a third shield. The dissection process was thus accomplished with operator radiation exposure limited to one kernel at a time. The dissected full-fat fractions were then equilibrated in room air overnight before the weights of the fractions were established. The vials were placed in a horizontal position to allow the samples better air contact. The weighed germ fractions from each successive vial were transferred and combined in a 120-mL screw-cap glass bottle containing 50 mL of hexane. Likewise, the endosperm–hull fractions were combined in a larger glass bottle containing 200 mL of hexane. The fractions were submerged in hexane to prevent unwanted microbial growth. These bottles were stored in the lead shield until approximately half the total corn sample had been dis-

Table II. Zinc-65 Activity, Percent Zinc Abundance, and Comparative Yields of Tissues from Corn Plants Grown in Nutrient Solution

Tissue	^{65}Zn at t_0		^{65}Zn abundance in plants, %	Nonradioactive zinc abundance, in plants,[a] %	Weight distribution of plant tissues	
	μCi	μCi/g of tissue			Radioactive, %	Nonradioactive,[a] %
Leaves						
Upper	1442	481			0.7	
Middle	1810	151			2.7	
Lower	1242	69			4.0	
Total tissue	4494	136	24.8	18.0	7.4	9.8
Stem sections						
1 (tassels)	643	129			1.1	
2	747	25			6.8	
3	317	10			7.0	
4	351	6			12.9	
5	1066	14			17.4	
Total tissue	3124	16	17.3	17.3	45.2	33.2
Roots	3907	55	21.6	27.3	16.0	15.0
Husks	666	48	3.7	3.3	3.2	3.7
Cobs	909	29	5.0	6.2	7.0	9.1
Kernels	4982	53	27.6	27.9	21.2	29.2

[a] Results according to Clark (1975b).

sected and collected before grind-defat operations were begun.

Grinding and Defatting. In a hood, the full-fat germ and endosperm–hull fractions representing one-half the total sample were ground and simultaneously defatted in a stainless-steel grinding chamber in hexane in a series of three separate 15- and 30-min grind operations, respectively. The endosperm–hull fractions required longer grind periods and lower initial grind speeds for the first 5 min because of the relatively greater amount of energy required to grind this fraction. After each grind operation and a settling period of 10 min, the supernatant hexane–oil mixture was transferred to a screw-cap 250-mL polypropylene centrifuge bottle for centrifugation for 10 min. This hexane–oil mixture was then filtered into a 250-mL Phillips beaker through a glass wool pledget to separate out minute particulate material in suspension.

The filtered hexane from all the extractions was continually removed by air circulation in the hood until a volume of about 150 mL remained. This resultant hexane–oil mixture was then filtered a second time through a No. 1 filter paper. Hexane was further removed by evaporation until only the oil fraction remained. The oil fraction was weighed to establish recovery.

The ground and defatted germ and endosperm–hull fractions were quantitatively transferred with hexane from the grinding chamber to a 250-mL centrifuge bottle. After centrifugation, the hexane washes were combined with the hexane–oil mixture previously described. The remaining traces of hexane were removed from the residual ground material in the centrifuge bottle by air circulation. The fractions were weighed in their respective bottles to establish recovery.

Decontamination. Apparatus was decontaminated by immersion in a hot solution of 2% w/v NaEDTA-2% w/v Na_2CO_3, with the exception of grinding chamber cutter bearings, which retained radioactivity after cleaning and were disposed of by standard procedures.

RESULTS AND DISCUSSION

Translocation and Distribution of ^{65}Zn in Corn Plants. Of the total ^{65}Zn supplied (24.97 mCi) via the nutrient solution, 92.1% was recovered in the experiment and 78.6% of the total ^{65}Zn was taken up by the two corn plants. Table II shows that the decreasing order of ^{65}Zn activity that accumulated in the specific tissues was: kernels (4982 μCi), leaves, roots, stems, cobs, and husks (666 μCi). However, the zinc concentrations were the highest in the leaves and in the tassel section of the stems where the activities (μCi/g tissue) were the highest. The leaf and tassel components represent only a minor part of the total plant weight.

Comparative results in Table II show that the yields of the individual radioactive tissues derived from this study were similar to yields for nonradioactive tissues acquired from the same corn variety and grown in a closely similar nutrient solution (Clark, 1975b), except that the radioactive stems represented 45.2% of the total plant, whereas the nonradioactive stems represented only 32.7% of the total plant. The radioactive tissues were air-dried at room temperatures; the nonradioactive tissues were dried in a forced-air oven at 70 °C.

The concentrations of zinc in tissues of corn plants grown in nutrient solution were reported to range from a high of approximately 40 ppm in leaves and roots to an average level of approximately 20 ppm in husks, cobs, sheaths, stalks, tassels, and kernels, on a dry weight basis (Clark, 1975b). Using these values and the tissue weights reported by Clark, we calculated the percent abundance of zinc in the corn tissues he described (Clark, 1975b). Then we compared the percent abundance values he obtained with those for ^{65}Zn distribution (Table II). The values obtained by our radiometric analysis and his emission spectrographic techniques compare very closely. By both measurement techniques over 27% of the zinc in the plant was incorporated into the kernels.

Depletion of ^{65}Zn from the nutrient solution was rapid, especially at the time the fourth and fifth doses were supplied at 64 and 71 days after transplanting. The radioisotope accumulated preferentially in the tassels, where localized concentrations were expected to occur (Ulrich, 1952), and in the leaves. The relatively greater movement of ^{65}Zn into the leaves reflects the continued development of leaf tissues after tasseling and acropetal transport of zinc from roots to leaves in the transpiration stream (Russell and Barber, 1960). The ^{65}Zn concentration was greater in kernels than in cobs and probably reflects some redistribution of zinc during kernel maturation (Williams, 1955).

In our study, delaying radioisotope application until tassel emergence reduced the quantity of ^{65}Zn required and still permitted adequate labeling of the kernels. However, considering the pattern of zinc translocation and accu-

Table III. Summary of Fractionated Radioactive (Zinc-65) and Nonradioactive Whole Kernel Corn

Corn sample	Radioactive (^{65}Zn)	Nonradioactive
Whole kernel, g	92.146	104.109
No. of kernels	418	460
g/kernel	0.2204	0.2263
Dissected full-fat fractions		
Germ, g	10.374	13.202
g/kernel	0.0248	0.0287
% germ	10.20	11.29
Endosperm-hull, g	91.078	103.470
g/kernel	0.2179	0.2249
% endosperm-hull	89.80	88.71
Total germ + endosperm-hull, g	101.452[a]	116.682[a]
g/kernel	0.2427	0.2537
Defatted fractions and oils		
Germ, g	6.650	8.030
g/kernel	0.0159	0.0175
% germ	6.73	7.28
Endosperm + hull, g	87.540	98.40
g/kernel	0.2094	0.2139
% endosperm-hull	88.57	89.17
Germ oil, g	2.541	2.233
% germ oil	2.57	2.02
Endosperm-hull oil, g	2.107	1.683
% endosperm-hull oil	2.13	1.53

[a] Total weight of full-fat fractions increased due mostly to moisture content increase as a result of steeping.

Table IV. Composition of Nonradioactive Whole-Kernel Corn Sample (Dry Weight Basis)

% protein	15.2[a]
% starch	67.2[b]
(% amylose in starch)	28.5[b]
% pentosans	6.7[c]
% fat	3.6[b]
% ash	1.3[c]
% fiber	2.2[c]
μg of Zn/g	28.8[b]

[a-c] Represent the average of nine, three, and two determinations, respectively.

Table V. Activity of Zinc-65 in Corn Fractions

Fraction	μCi ^{65}Zn at t_4[a]	μCi ^{65}Zn/g of tissue at t_4
Defatted germ flour	1458.70	219.35
Defatted endosperm-hull flour	1206.90	13.79
Oil from germ	20.96	8.25
Oil from endosperm-hull	2.48	1.18

[a] t_4 = 209 days after t_0.

mulation in the kernels and the ontogeny of the plant, a more efficient labeling treatment might have been to delay treatment until full anthesis and to supply smaller quantities of the radioisotope for 4 rather than 5 weeks. If this procedure were followed, it probably would reduce excessive labeling of the leaves acropetal to the ear, and it would synchronize better the radioactive zinc supply with the period of grain growth (Duncan, 1975).

Corn Fractionation Yields and Recoveries. Recovery and yield data for fractionated radioactive and nonradioactive corn kernels are given in Table III. Kernel size did not differ significantly between radioactive and nonradioactive kernels. The total weight of the full-fat fractions was 10 to 12% greather than the original weight present in the whole kernel corn. This increase was probably due to moisture absorption during steeping.

In the kernels, the highest levels of minerals, including zinc, are found in the germ fraction, with considerably lower levels occurring in the endosperm fraction (Garcia et al., 1972). The full-fat germ is composed of significant quantities of (i) protein of high quality, (ii) the edible oil, (iii) carbohydrates that include sugars, starch, and pentosans, and (iv) mineral constituents, principally as phytates. However, the endosperm–hull fraction that represents the major part of the kernel is composed largely of carbohydrates, with starch as the main constituent.

The composition of the nonradioactive whole kernel corn is given in Table IV. Comparable analyses were not performed on the radioactive corn kernels because the entire sample was fractionated into germ, endosperm–hull, and oil fractions.

Distribution of ^{65}Zn. After dissecting the kernels the distribution of the total ^{65}Zn in the full-fat fractions that were submerged in hexane was established by ratemeter readings. Although the full-fat germ represented only 10.23% of the radioactive whole-kernel weight, it contained 56.38% of the ^{65}Zn activity. In contrast, the endosperm–hull fraction represented 89.77% of the corn sample weight and contained 43.62% of the ^{65}Zn activity. Thus, the zinc had accumulated preponderantly in the phytate-rich germ fraction.

Radiometric quantitation revealed that 97.50% of the ^{65}Zn present initially in the whole kernel sample was recovered in the four final fractions from the kernels. Figure 1 presents the percent distribution of ^{65}Zn in these fractions and it also reveals that 1.14% of the ^{65}Zn was retained on filters and cutter bearings. In addition, 1.36% of the ^{65}Zn activity was recovered in the NaEDTA + Na_2CO_3 solution, and no radioactivity was recovered in the steep water.

The half-life of ^{65}Zn (245 days) is long enough to permit its incorporation into plants followed by tissue fractionation to produce products for subsequent experiments. Figure 2 depicts the time schedule showing the major processes in the experiment and, in addition, relates the fractional radioactivity of the tracer remaining at four different times when quantitation measurements were made. From this figure, it can be seen that the ^{65}Zn activity of the defatted germ, endosperm–hull, and oil fractions was determined at t_4 when the fractional radioactivity remaining represented 0.5536 of that initially present at t_0 (7-1-75).

The activities of the four corn fractions at t_4 are shown in Table V. Relative activities of ^{65}Zn in the products

Table VI. Relative Abundance of Zinc in Fractions of Corn as Measured by Radiometric and Atomic Absorption Techniques

Zinc-65 labeled corn and fractions			Commercial corn products		
Product	Activity, t_0, μCi ^{65}Zn/g of tissue	Relative abundance	Product	μg Zn/g	Relative abundance
Whole kernel corn	54.07	1.00	Whole kernel corn	22.9	1.00
Full-fat germ	270.71	5.01	Defatted corn germ flour	208.0	9.08
Full-fat endosperm-hull	23.87	0.44	Yellow corn meal	11.0	0.48
Defatted germ	396.17	7.33	Refined corn oil	0.58	0.025
Defatted endosperm-hull	24.91	0.46	Dark corn syrup	0.28	0.012
Oil from germ	14.90	0.28	Light corn syrup	0.15	0.006
Oil from endosperm-hull	2.13	0.039			

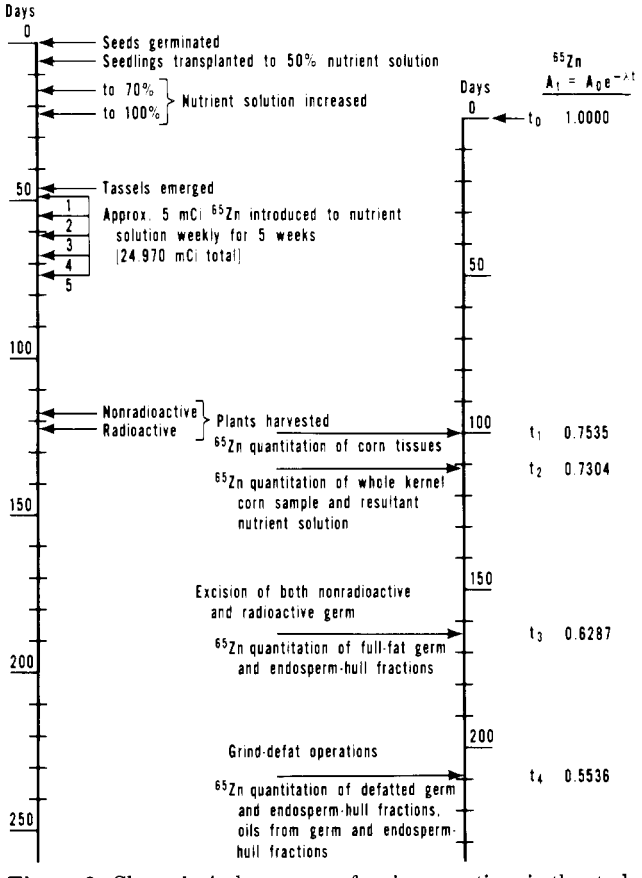

Figure 2. Chronological sequence of major operations in the study and their relationship to the radioactive decay of ^{65}Zn.

ranged from a high of 219 in the defatted corn germ flour to a low of 1 in the oil derived from the full-fat endosperm–hull fraction. Concentrations of mineral and trace elements are known to vary considerably in corn-based food products, depending on the kernel tissue involved and the degree that processing has fractionated or modified these tissues (Garcia et al., 1974a). For example, corn meal (an endosperm product) and a prepared defatted corn germ flour averaged approximately 10 and 200 ppm zinc, respectively. Zinc in other corn-based food products tested ranged from more than 40 to less than 0.2 ppm for whole kernel popcorn and light corn syrup, respectively. Not only does the zinc content of grain fractions vary widely, but different grain fractions contain different amounts of phytates, which have been implicated in adversely affecting the bioavailability of zinc (O'Dell and Savage, 1960; Oberleas et al., 1962; Welch et al., 1974).

Sensitive analytical methods have been developed to determine the levels of heavy metals, including zinc, present in corn tissues and corn-based food products intended primarily for human consumption (Clark, 1975a,b; Garcia et al., 1974b). Although the content of trace elements has been quantitated, knowledge of the bioavailability of these important constituents is fragmentary, and assessment can be complex.

Some form of processing or milling is required for cereals before products are prepared for human consumption. The wet or dry milling processes remove the mineral-rich germ fraction of corn which contains the phytates, while leaving behind the major portion of the kernel. This is preponderantly an endosperm product, but it contains only minor quantities of minerals and zinc. The dry-milled product is the starting material for a wide variety of processed corn foods that include many breakfast and snack foods.

Until recently, after the corn oil was extracted from the germ fraction, the resultant defatted germ material was combined with other corn fractions for use primarily for animal feed purposes. However, a food-grade defatted corn germ flour has been developed (Blessin et al., 1973) that contains over 20% protein of high quality; because of its bland taste, this flour can be incorporated into a wide variety of baked goods.

The relative abundance of zinc in the labeled corn fractions measured radiometrically as ^{65}Zn is compared with the relative abundance of nonradioactive zinc in selected commerical corn products in Table VI. Our radiometric assessment compares favorably with values previously reported for corn tissues and corn-based food products (Clark, 1975a,b; Garcia et al., 1974b). Determination of the bioavailability of zinc in the fractions that we prepared should give an indication of the zinc availability in consumer products made with comparable corn fractions. By using endogenously labeled corn fractions in such studies, the bioavailability of zinc can be determined unambiguously.

ACKNOWLEDGMENT

We thank W. R. Findley for supplying the seed corn, M. Blankendaal for technical assistance, G. W. Evans and J. L. Sell for use of their isotope quantitation facilities, and Dora L. Miller and C. C. Harris for compositional analyses of nonradioactive samples.

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Received for review February 28, 1977. Accepted August 2, 1977. The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

Composition of the Essential Oils of Sudangrass and Hybridsorgo, Forage Sorghums

Takayasu Kami

The essential oil of Sudangrass, a grass sorghum, was isolated by steam distillation of the fresh grass with a yield of 0.0006%. The essential oil was fractionated to each functional group, and the fractions were analyzed by gas chromatographic comparison with authentic samples using polar and nonpolar columns. Thus, 62 compounds including 13 acids, 6 phenols, 5 aldehydes, 6 ketones, 6 alcohols, 10 esters, and 16 hydrocarbons were tentatively identified as steam-distilled Sudangrass constituents. Almost all the compounds, however, have been detected in the essential oil of Hybridsorgo. In order to clarify the odor difference, quantitative analyses were further carried out on both essential oils of Sudangrass and Hybridsorgo: Sudangrass was rich in carbonyl compounds, while Hybridsorgo was rich in phenols.

In a previous investigation, the essential oil of Hybridsorgo had been fully analyzed by means of combined gas chromatography–mass spectrometry (GC–MS) to identify the aromatic constituents (Kami, 1975). The present work deals with the quantitative analyses of the essential oils of Sudangrass and Hybridsorgo by means of gas chromatography (GC).

Forage sorghums are classified to six agricultural species, and many varieties in each species are cultivated all over the world as feedstuffs of domestic animals. Among them, Hybridsorgo and Sudangrass are especially cultivated in the southwestern warm district of Japan as roughages of dairy cattle, because of their strong drought resistance, strong regrowth, and higher forage yield than other forage grasses. Of the two, Hybridsorgo is superior in yield (leaf size, leaf number, stalk diameter, and plant height), moisture percent, and sugar content, while Sudangrass is superior in offshoot (Harada et al., 1966). In Japan, Hybridsorgo is generally believed to be inferior in its palatability for dairy cattle.

EXPERIMENTAL SECTION

Materials. Sudangrass (Piper) was cultivated on a farm of the Faculty of Fisheries and Animal Husbandry, Hiroshima University, and the grass was harvested by mower in Oct 1970 as the second crop.

In the gas chromatographic analysis of Hybridsorgo, the essential oil obtained in a previous experiment was used (Kami, 1975).

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Isolation of Essential Oil from Sudangrass. The fresh crop (160 kg) was chopped and steam distilled according to a procedure previously described (Kami, 1975) to give about 72 L of a cloudy distillate in a water-cooled trap, and 3.4 g and 0.8 g of colorless aqueous condensates in ice–water-cooled and dry ice–acetone-cooled traps, respectively. The cloudy distillate of the water-cooled trap was saturated with sodium chloride, and 2-L lots were then extracted twice with 300 mL of redistilled diethyl ether to yield a dark brown oil with a sweet silage-like odor (0.96 g, η^{20}_{D} 1.4470, pH 3.2). The essential oil was stored in a sealed glass tube at 3 °C, as were the aqueous condensates from the ice–water- and dry ice-cooled traps.

Fractionation of the Essential Oil. A portion (680 mg) of the essential oil was sequentially extracted (Kami, 1975) to separate acid (brown viscous, 42 mg), phenolic (light brown viscous, 31 mg), and basic (drab viscous, 22 mg) fractions. The remaining neutral oil layer (302 mg) was transferred to 100 mL of *n*-pentane in a 200-mL beaker and stirred with 10 g of Mallinckrodt 100-mesh silicic acid (freshly activated at 125 °C for 3 h just before use) at room temperature for 2 h on a magnetic stirrer. The silicic acid, after being filtered out on a filter paper, was stirred with 100 mL of diethyl ether at room temperature for 2 h to dissolve the adsorbed polar components. The ethereal solution and the pentane filtrate thus obtained were each dried over anhydrous sodium sulfate, filtered, and evaporated on a rotary evaporator at room temperature, yielding polar (yellow brown liquid, 150 mg) and nonpolar (white crystal, 23 mg) fractions.

GC of the Unfractionated Essential Oil and the Acid, Phenolic, Polar, and Nonpolar Fractions. An FID-type Yanagimoto GCG-550T apparatus was operated